New and Notable

Breaking the Millisecond Barrier: Single Molecule Motors Wobble to Find their Next Binding Sites

Christopher L. Berger*
Department of Molecular Physiology & Biophysics, University of Vermont, Burlington, Vermont

Over the last 20 years, advances in single molecule biophysics have allowed the structure and dynamics of biological macromolecules to be examined at increasingly high resolution (1). The study of motor proteins in particular has benefitted tremendously from the development of numerous optical techniques for examining both the mechanical (2,3) and structural changes (4–9) required for biological motility at the molecular level. But as the spatial resolution in single-molecule fluorescence experiments has improved to the nanometer level, this has somewhat been at the expense of temporal resolution, which has remained on the millisecond timescale. While millisecond time resolution is more than adequate for detecting changes in position or orientation between stable conformations of the protein of interest, important dynamic information during the transitions between these states, which occur on the microsecond timescale, are lost. Beausang et al. (10) overcome this temporal limitation in a report in this issue of *Biophysical Journal*, by observing brief changes in the microsecond wobble of its lever arm as myosin V steps along actin filaments using single-molecule fluorescence polarization in a total internal reflection fluorescence (TIRF) microscopy assay (polTIRF).

Unlike improvements in spatial resolution that have relied on the ability to collect more photons, the authors achieve this significant breakthrough in time-resolved structural studies at the single-molecule level by collecting fewer photons. The 50-fold improvement in time resolution relative to previous polTIRF measurements from the same group (4,5,7,11) was achieved by being able to switch the polarization of the excitation light every 100 μs instead of 5–10 ms, and by moving to a single-photon counting detection system. However, the improved time-resolution results in an unavoidable reduction in signal/noise that necessitates corresponding improvements in the ability to discern changes in polarization output. The authors solve this problem by implementing an innovative analysis scheme, using a maximum-likelihood, multitrace change-point algorithm previously developed in their lab (12).

Thus, beyond the improvements to the polTIRF instrumentation presented here, Beausang et al. (10) provide a blueprint for future advances in other time-resolved, single-molecule structural studies, involving a combination of enhancements in both the hardware and software components of data acquisition and analysis. In their work, this powerful new approach has resulted in elucidation of a number of new observations about the myosin V stepping mechanism, including what is believed to be the first direct observation of rotational wobble of the myosin V motor domain as it thermally searches for the next available actin binding site (Fig. 1 *b*) between stable attachments to the actin filament before (Fig. 1 *a*) and after the step (Fig. 1 *c*). While this wobble was predicted from earlier optical trap experiments (13) and detected from lateral fluctuations of a relatively large gold particle attached to the lever arm (14), these experiments used a small organic fluorescent probe to demonstrate the high-microsecond rotational mobility of the lever arm when it is detached from actin. Given the increasing recognition of the importance of thermal fluctuations in macromolecular function, the instrumentation and analysis techniques developed by Beausang et al. (10), extending measurements of single-molecule structural dynamics into the microsecond timescale, open up exciting new avenues of investigation for researchers working with a number of different biological systems.

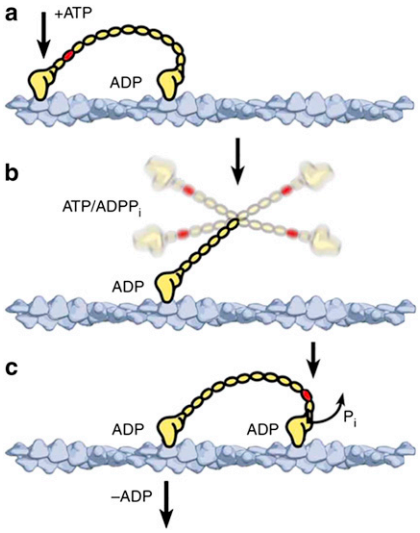

FIGURE 1 Schematic of myosin V stepping along an actin filament. (*a*) Upon binding ATP the trailing head detaches and (*b*) undergoes a high mobility thermal search for the next available binding site (the microsecond-scale rotational wobble directly observed in the report by Beausang et al. (10) in this issue of *Biophysical Journal*), where (*c*) it binds stably, allowing for product release and the associated powerstroke (adapted from Dunn and Spudich (14)).

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*Correspondence: cberger@uvm.edu

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